Lipids of *Viburnum opulus* seeds

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Lipid components of the seeds of *Viburnum opulus* (*Caprifoliaceae* family) were investigated. The neutral lipids consist of eight classes, the glycolipids consist of three classes, and the phospholipids contain seven classes. The fatty-acid contents of all of the acyl-containing lipids were determined. The 18 : 2 fatty acid is the main component of all the lipid fractions. The content of saturated acids is greater in the glycolipids and phospholipids. The lipophilic components, higher fatty alcohols and sterols, were identified.

Key words: *Viburnum opulus*, *Caprifoliaceae*, seeds, lipids, guelder rose.

Guelder rose (*Viburnum opulus L.*, the honeysuckle family, *Caprifoliaceae*) is a plant widely distributed almost over the whole territory of Russia.

The bark and fruit of guelder rose have found application in medicine.[1] Iridoids, triterpene saponins, catechols, hydroxycoumarins, and flavonoids have been isolated from its bark. Anthocyans, catechols, chlorogenic acid, pectins, carbohydrates, triterpenoids, polysaccharides, and organic acids have been found in the fruits.[1–5] Guelder rose fruits are used in food production, but the seeds are discarded as wastes.

Data on the oil content of the seeds and their overall fatty-acid composition have been reported.[6,7] The oil from the guelder rose seeds stimulates the reparative regeneration of skin and stomach mucose.[8]

Our study deals with guelder rose seeds. We have studied neutral (NL) and polar lipids (PL), including glycolipids (GL) and phospholipids (PhL). The analysis was performed with powdered air-dried seeds. Neutral lipids were isolated by extraction with petroleum ether (specimen 1) or dichloromethane (specimen 2) (the latter solvent is often used to extract oils in pharmaceutical industry). In both cases, the oil was yellow-orange colored. However, in the former case, it was labile and transparent, whereas in the latter case, it was thick and nontransparent and formed a precipitate on storage. The data on the oil and carotenoid contents in both samples are presented in Table 1.

Table 1. Some characteristics of *Viburnum opulus* seeds

Specimen	Oil content relative to totally anhydrous substance (%)	Content of carotenoids /mg g^{-1}	Moisture content (%)
1	11.7	0.054	5.1
2	14.7	0.096	5.1

The higher content of carotenoids in specimen 2 might be due to the fact that dichloromethane also extracts oxidized carotenoids, xanthophylls, from the plant raw material. The increase in the oil content is due to the extraction of non-lipid substances, in particular, pigments, from the plant raw material. Pharmocological assays of both specimens for reparative regeneration of skin and stomach mucosa did not reveal noticeable differences between their biological activities; therefore, subsequently NL were isolated from the raw material using petroleum ether.

The resulting NL were separated into individual classes by column chromatography on silica gel (SiO_2) (Table 2). It can be seen from the table that saponifiable components predominate.

Mild alkaline hydrolysis of acyl-containing lipids gave fatty acids (FA), which were identified by GLC as the corresponding methyl esters (Table 3). Thus, the guelder rose oil can be classified as highly unsaturated (up to 97% unsaturated FA). The ratio of the 18 : 1, 18 : 2, and 16 : 0 acids in this oil is close to that in vegetable oils such as sunflower and corn oils.

Higher fatty alcohols and free sterols account for 1% of guelder rose oil. The IR spectra of higher fatty alcohols exhibit an absorption band at 3200—3600 cm^{-1} corresponding to associated hydroxyl groups. When the sample was treated with AcCl, this IR band disappeared, and a band at 1750 cm^{-1}, due to the ester group appeared instead. The higher fatty alcohols were further identified by the GC/MS method. The results obtained indicate that this fraction consists mostly of nonbranched alcohols: tetracosan-1-ol, hexacosan-1-ol, and octacosan-1-ol, whose contents in the total alcohol fraction are 19.4, 59.0, and 17.1%, respectively. The minor components are represented by docosan-1-ol (0.3%), pentacosan-1-ol (2.8%), and heptacosan-1-ol (0.7%).

The overall mass spectrum recorded on an MX-1320 instrument with direct injection of the sample into the

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Table 2. Composition of lipids from *Viburnum opulus* seeds

Classes of lipids	Contents (%)
Neutral lipids (NL)	11.7[a]
Carotenoids	Traces[b]
Esters of fatty alcohols and sterols	Traces[b]
Triacylglycerols (TAG)	92.7[b]
Free fatty acids (FFA)	2.7[b]
High-molecular-weight fatty alcohols (HFA)	0.4[b]
Diacylglycerols (DAG)	3.0[b]
Sterols	0.6[b]
Monoacylglycerols (MAG)	0.6[b]
Glycolipids (GL)	0.3[a]
Monogalactosyldiacylglycerols (MGDG)	41.6[c]
Sterylglycosides (SG)	30.6[c]
Digalactosyldiacylglycerols (DGDG)	27.8[c]
Phospholipids (PhL)	0.1[a]
N-acylphosphatidylethanolamine (*N*-acyl PE) + NC[e]	27.1[d]
Phosphatidic acid (PA)	12.0[d]
Cardiolipin (CL)	9.2[d]
Phosphatidylethanolamine (PE)	18.4[d]
Phosphatidylcholine (PC) + Phosphatidylinositol (PI)	28.5[d]
lyso-Phosphatidylcholine (l-PC)	4.8[d]

[a] Of the weight of air-dried raw material. [b] Of the weight of NL. [c] Of the weight of GL. [d] Of the weight of PhL. [e] NC is a nonidentified component.

Table 3. Fatty acid composition of acyl-containing classes of neutral lipids of *Viburnum opulus* seeds (%)

Acid	NL	TAG	FFA	DAG	MAG
12 : 0	Traces	Traces	Traces	0.6	2.6
14 : 0	0.5	0.1	Traces	0.8	2.7
16 : 0	2.0	1.9	4.0	3.7	4.0
16 : 1	Traces	Traces	0.3	0.5	0.7
18 : 0	0.6	0.5	1.0	0.9	1.0
18 : 1	42.5	44.3	32.0	36.9	36.9
18 : 2	52.6	51.7	57.6	53.6	49.9
18 : 3	1.8	1.5	5.1	3.0	2.2
Total saturat. FA	3.1	2.5	5.0	6.0	10.3
Total unsaturat. FA	96.9	97.5	95.0	94.0	89.7

ion source exhibits resolved peaks for ions of minor high-molecular-weight components: miricyl alcohol (hentriacontan-1-ol), $C_{31}H_{64}O$, *m/z* 452 $[M]^+$, 434 $[M-18]^+$, 406 $[M-18-28]^+$, *etc.* $[M-18-n28]^+$ and tetratriacontan-1-ol, $C_{34}H_{70}O$, *m/z* 494 $[M]^+$, 476 $[M-18]^+$, 448 $[M-18-28]^+$, *etc.* $[M-18-n28]^+$.

The mass spectra of all the above-listed alcohols are similar.[9–12] The region of low masses contains intense peaks with *m/z* 41, 43, 55, 57, 69, 71, 83, 85, 97, 111, and 125. The peak intensity decreases as the mass number increases. Molecular ion peaks are virtually absent. The peaks of ions resulting from abstraction of a water molecule, $[M-18]^+$, and then an ethylene molecule, $[M-18-n28]^+$, are relatively weak; their intensity is less than a percent of that of the highest peak. The peak of the $[M-18]^+$ ion is normally somewhat higher than the $[M-18-28]^+$ ion peak. The subsequent ejections of ethylene molecules account for even less intense peaks.

The GC/MS analysis of the fraction of free sterols indicates the presence of three main components. The major component is β-sitosterol (50.6%; $[M]^+$ 414). Its mass spectrum is almost identical to that reported in the literature.[13] The same is true for campesterol (3.4%; $[M]^+$ 400). The third component with *m/z* 412 (7.5%) can be either stigmasterol, or isofucosterol, or avenosterol. The second most intense peak (75%) in the resulting mass spectrum corresponds to an ion with *m/z* 314. According to the published data,[13] the intensity of this peak in the spectrum of stigmasterol is 7%, that in the spectrum of avenosterol is 40%, and in the case of isofucosterol, it is 76%. In view of these data, the third component is most likely isofucosterol. This is also indicated by the intensity of the peak with *m/z* 271. According to the published data,[13] this peak is not exhibited in the spectrum of stigmasterol; in the case of isofucosterol, its intensity is 7%, and for avenosterol, it is 60%. In our case, the intensity of this peak is 8%. In addition, we found compounds whose molecular weight differed from that of the three above-mentioned sterols by 18 atomic units. In each case, several compounds with the same molecular weight but different chromatographic retention times were detected. In all probability, these compounds result from dehydration of the corresponding sterols during chromatography (see Experimental). The possible products of dehydration of sitosterol amount to ~24.7%, those derived from isofucosterol amount to 5.3%, and those formed from campesterol are 1.5%. The sterol fraction was found to contain as well a compound with $[M]^+$ 410 (5.4%) exhibiting a mass spectrum typical of sterols. As for other sterols, a compound (1.6%) whose molecular weight differed by 18 atomic units, was also detected.

To isolate PL, the residue after the removal of NL was extracted with a $CHCl_3$—MeOH mixture. Column chromatography on SiO_2 gave PL (0.5% w/w of the air-dried raw material). To separate PhL from GL, preparative TLC and reprecipitation from acetone were used. Carbohydrates were removed by column chromatography on Molselect G-25. Thus, 0.3% of GL and 0.1% of PhL with respect to the weight of air-dried seeds were isolated. By using one-dimensional and two-dimensional TLC, seven components were detected in the PhL fraction; each of them exhibited positive reaction for phosphorus. Preparative TLC on SiO_2, after purification and rechromatography, gave six components. Comparison of

the chromatographic mobilities of the samples studied with those of the model compounds and with published data, qualitative reactions, and the results of alkaline and acid hydrolysis followed by identification of the resulting acids and amino alcohols made it possible to detect seven PhL classes (Table 2). The fatty-acid composition of PhL differs from that of NL both in the set of FA present and in their ratios in individual fractions (Table 4). Acids of the composition 15 : 0, 15 : 1, 16 : 2, and 17 : 1 were additionally found in the PhL fraction. It is noteworthy that the content of saturated acids practically in all PhL fractions is relatively high, compared to that in NL in which the highest content of these acids was found in monoacylglycerols (10%, Table 3). This is due to the substantially higher content of the 16 : 0 acid almost in all the PhL classes. However, as in the case of NL, the 18 : 2 acid predominates in all PhL fractions; its content is comparable to that of the 16 : 0 acid only in the lyso-phosphatidylchloline.

Using the method of column chromatography and preparative TLC on SiO_2, three fractions were isolated from GL. Based on a comparison of their TLC behavior with that of model samples and on qualitative reactions, they were tentatively classified as monogalactosyldiacylglycerols, sterylglycosides, and digalactosyldiacylglycerols (Table 2).

Mild alkaline hydrolysis of monogalactosyldiacylglycerols and digalactosyldiacylglycerols afforded fatty acids, which were identified as the corresponding methyl esters (Table 5). The monogalactosyl- and digalactosylglycerols remaining after the removal of fatty acids were subjected to acid hydrolysis; in both cases, this yielded galactose, identified by GLC as the corresponding trimethylsilyl derivative.

Table 4. Fatty acid composition of acyl-containing classes of phospholipids of *Viburnum opulus* seeds (%)

Acid	Total PhL	*N*-acyl PE + NC*	PA	CL	PE	PC + PI	l-PC
12 : 0	0.5	0.2	0.9	1.2	2.0	1.6	1.7
14 : 0	0.5	0.4	1.1	1.3	0.7	0.9	0.6
15 : 0	—	0.5	0.4	0.2	—	0.2	0.2
15 : 1	—	0.7	—	—	—	0.6	—
16 : 0	13.9	8.3	17.3	25.6	12.0	17.3	31.7
16 : 1	0.6	0.8	1.0	1.3	1.0	1.2	2.9
16 : 2	—	—	—	—	—	—	3.2
17 : 1	—	0.7	—	—	—	2.1	—
18 : 0	2.6	1.8	2.3	5.8	4.8	3.9	5.8
18 : 1	24.7	27.1	25.8	18.0	24.4	23.0	17.3
18 : 2	55.2	57.6	48.9	42.9	50.2	46.0	34.4
18 : 3	2.0	1.9	2.3	3.7	4.9	3.2	2.2
Total saturat. FA	17.5	12.2	22.0	34.1	19.5	23.9	40.0
Total unsaturat. FA	82.5	88.8	78.0	65.9	80.5	76.1	60.0

* NC is an nonidentified component.

Table 5. Fatty acid composition of acyl-containing classes of glycolipids of *Viburnum opulus* seeds (%)

Acid	MGDG	DGDG
14 : 0	2.1	2.3
15 : 0	—	0.7
16 : 0	10.9	13.6
16 : 1	Traces	1.2
17 : 1	1.4	1.0
18 : 0	2.4	4.8
18 : 1	31.8	33.8
18 : 2	46.5	37.6
18 : 3	1.5	Traces
20 : 0	3.4	5.0
Total saturat. FA	18.8	26.4
Total unsaturat. FA	81.2	73.6

Unlike the acyl-containing fractions of NL and PhL, the GL fraction was found to contain the 20 : 0 acid. Regarding the proportions of particular acids, monogalactosyldiacylglycerols and digalactosyldiacylglycerols are similar, except for the 18 : 2 acid, whose content in monogalactosyldiacylglycerols is much higher. As in the case of acyl-containing classes of PhL, in this case, too, the content of saturated acids was found to be higher than that in NL, mostly at the cost of the 16 : 0 acid. The same tendency in relation to palmitic acid has been noted in a previous study[14] dealing with the acyl-containing fractions of GL from the seeds and seed vessel of *Elaegnus angustifolia L.* (narrow-leaved oleaster) in which the content of the 16 : 0 acid in the corresponding classes of GL was found to be higher than that in the same fractions of NL.

The fraction that was tentatively assigned to sterylglycosides showed positive reactions for carbohydrates (α-naphthol,[15] aniline phthalate[16]) and for sterols (20% $HClO_4$, 50% H_2SO_4).[16] The IR spectrum of the fraction exhibited intense absorption bands at 1100—1200 and 3200—3600 cm^{-1}. After acid hydrolysis under rigorous conditions, no changes were observed. Oxidation of the fraction with periodic acid gave a product that showed a positive reaction for sterols (anisaldehyde, vanillin—H_3PO_4), and its TLC mobility corresponded to that of a model specimen of natural sterols but did not exhibit analytical reactions for carbohydrates.

Thus, the results obtained permit the *Viburnum opulus* seeds to be classified as medium oil-bearing. Since the raw material contains a lot of pigments, which degrade the organoleptic properties of the oil, extraction conditions that make it possible to obtain an oil with low pigment contents were selected. Our study showed that the NL fraction consists mostly of unsaturated fatty acids and its fatty-acid composition is quite narrow; the fatty-acid composition of PL is much more diversified. An increased content of the 18 : 2 acid in all the PL classes was noted. The use of mass spectrometry and the GC/MS method for identification of lipophilic components made it possible to detect compounds that had not

been found previously among the lipids from *Viburnum opulus* seeds. In general, regarding its lipid and fatty-acid composition, the oil is close to food vegetable oils.

Experimental

Mass spectra were recorded on an MX-1320 spectrometer; GC/MS analysis was carried out on an HP 5890 chromatograph with an HP 5972 A mass selective detector (a 0.25 mm×30 m HP-5 chromatographic column, initial temperature 100 °C, heating rate 20 deg min^{-1}, final temperature 270 °C); the data were processed using an HP MS ChemStation, MX-1320; the ionizing voltage was 70 eV and 12 eV, and the temperature of the ionization chamber was 70—100 °C.

The GLC analysis of methyl esters of fatty acids was carried out on a Chrom-5 chromatograph (a 1.2×3 mm column, 5% PDEGS on Chromaton N-AW-DMCS, 180 °C, He as the carrier gas, 40 mL min^{-1}).

The GLC analysis of the silyl esters of saccharides was performed on a Chrom-5 chromatograph (a 1.2×3 mm column, 5% SE-30 on Chromaton N-AW-DMCS, temperature programming in the 50—300 °C, range, He as the carrier gas, 35 mL min^{-1}).

The guelder rose seeds were waste products from the manufacture of foodstuffs from guelder rose fruits and were received from the Bel'sky forestry.

Neutral lipids were extracted from air-dried seeds with petroleum ether (40—60 °C) by digestion at ~20 °C; the extraction was carried out exhaustively.

Polar lipids were isolated from the residue remaining after the removal of NL by extraction with a $CHCl_3$—MeOH (2 : 1) mixture at ~20 °C repeated three times, according to the procedure reported previously.[17]

Neutral lipids were separated and identified as described previously.[18] Analytical TLC on SiO_2 was performed using Silufol plates and solvent systems for NL;[18] preparative TLC was carried out using silica gel SL 5/40 μm with 13% gypsum and the same solvent systems.

Polar lipids were separated, purified, and identified using plates with silica gel and the following solvent systems (volume ratios are given). For PhL:

— $CHCl_3$—MeOH—25% NH_3, 65 : 25 : 4 (*1*);
— $CHCl_3$—Me_2CO—MeOH—AcOH—H_2O, 6 : 8 : 2 : 2 : 1 (*2*);
— Pr^iOH—25% NH_3—H_2O, 50 : 7 : 15 (*3*);
— AcOH—Pr^nOH—H_2O, 1 : 2 : 1 (*4*).

For GL:

system (*1*) (presented above); and
— $CHCl_3$—Me_2CO—MeOH—AcOH—H_2O, 65 : 20 : 10 : 10 : 3 (*5*).

The PL were freed from carbohydrates on a column packed with Molselect G-25 (100—320 μm), which had swelled for 8—10 h in the 90 : 10 : 1 $CHCl_3$—MeOH—H_2O solvent system; PL were eluted with the same system. The process was monitored by TLC in system *1*.

PhL were freed from GL by reprecipitation from cooled acetone by adding acetone (5 mL) to a solution of PhL (100 mg in 0.2—0.3 mL of $CHCl_3$). The mixture was stirred and cooled in an ice bath for 1 h. The resulting suspension was centrifuged for 3—5 min at 2500 rpm. The precipitate was washed twice with cold acetone (2 mL) as described above using centrifugation.

Alkaline hydrolysis of the acyl-containing fractions of lipids was carried out by a procedure described previously;[18] acid hydrolysis was performed by a known procedure.[19]

The oil and moisture contents were determined by a known procedure;[20] the content of carotenoids was determined as described in an earlier study.[21]

The products of hydrolysis of PhL were analyzed by TLC in systems *3* and *4*; the spots were visualized by a solution of ninhydrin and Dragendorff reagent.[19]

To prepare silyl ethers of sugars, the solutions obtained after acid hydrolysis of monogalactosyl- and digalactosylglycerols, were filtered, neutralized by a solution of Na_2CO_3, and concentrated. Pyridine (0.2 mL), hexamethyldisilazane (0.04 mL), and chlorotrimethylsilane (0.02 mL) were added to the dry residue (2 mg). The mixture was shaken for 30 s and concentrated, and the silyl ethers were extracted by diethyl ether.[22]

The oxidation of sterylglycosides (41.9 mg) was carried out in a methanolic solution by adding HIO_4 (0.048 g of HIO_4 in 0.3 mL of H_2O). The mixture was stirred for 2 h at ~20 °C, and cooled to 0 °C. $NaBH_4$ (17 mg) was added, and the mixture was stirred for 2 h at ~20 °C. Then 6 *N* HCl (0.13 mL) and diethyl ether (1 mL) were added. The mixture was stirred for 4 h at ~20 °C, and NaCl (21 mg) was added to it. The ethereal layer was separated, the aqueous layer was extracted three times with diethyl ether, and the combined ethereal extracts were dried with Na_2SO_4 and concentrated to dryness.

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